

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1-12. (canceled).

13. (currently amended) A method for determining whether a selected DNA molecule encodes a gene expression region, said method comprising:

(Aa) obtaining RNA transcripts from an organism which comprises said selected DNA molecule, and

(Bb) screening said RNA transcripts for an RNA transcript corresponding ~~that is complementary to a selected portion of~~ said selected DNA molecule, wherein the nucleotide sequence of said selected portion of said selected DNA molecule is known, to thereby determine whether said selected portion of said selected DNA molecule encodes a gene expression region, wherein said screening comprises:

(ie) amplifying the RNA transcripts using a first oligonucleotide primer and a second oligonucleotide primer, wherein said first primer is complementary to a sequence of at least 10 continuous nucleotides located at or near the 3'-end of said selected portion of said selected DNA molecule, and said second primer is homologous to a sequence of at least 10 continuous nucleotides located at or near the 5'-end of said selected portion of said selected DNA molecule, wherein said amplifying comprises: and

(a) forming a RNA-DNA duplex comprising one of said RNA transcripts and a complementary DNA molecule adhered thereto, said duplex is formed by synthesizing a first DNA molecule complementary to at least a portion of one of said RNA transcripts using (1) said first oligonucleotide primer to prime synthesis of said first DNA molecule, (2) RNA-dependent DNA polymerase and (3) one of said RNA transcripts as a template, to thereby form an RNA-DNA duplex,

(b) preparing a single-stranded DNA molecule from said RNA-DNA duplex of (a) by hydrolyzing the RNA transcript of said RNA-DNA duplex using ribonuclease H,

(c) forming a doubled-stranded DNA molecule comprising the single-stranded DNA molecule of (b) and a complementary DNA molecule thereto, said doubled-stranded DNA molecule is formed by synthesizing a second DNA molecule complementary to at least a part of said single-stranded DNA molecule of (b) using (1) said second oligonucleotide primer to prime the synthesis of said second DNA molecule, wherein said second primer further comprises an RNA-transcriptable promoter sequence at its 5'-end, (2) DNA-dependent DNA polymerase, and (3) the single-stranded DNA molecule of (b) as a template, to thereby form a double-stranded DNA molecule,

(d) forming an RNA transcription product from said double-stranded DNA molecule of (c) using RNA polymerase, wherein RNA transcription is primed from the RNA-transcriptable promoter sequence, and

(e) repeating steps (a) to (d) using said RNA transcription product of
(d) as a template for the formation of the RNA-DNA duplex of (a), and
(iid) detecting an amplification product of (ie) corresponding complementary to
said selected portion of said selected DNA molecule, to thereby screen said RNA
transcripts for an RNA transcript that corresponds to said selected portion of said selected
DNA molecule, and
(C) repeating (A) and (B) on at least one other selected portion of said selected DNA
molecule.

~~wherein said amplifying comprises:~~

~~(e) forming a RNA-DNA duplex comprising one of said RNA transcripts and a~~
~~complementary DNA molecule adhered thereto, said duplex is formed by synthesizing a first~~
~~DNA molecule complementary to at least a portion of one of said RNA transcripts using (1) said~~
~~first oligonucleotide primer to prime synthesis of said first DNA molecule, (2) RNA dependent~~
~~DNA polymerase and (3) one of said RNA transcripts as a template, to thereby form an RNA-~~
~~DNA duplex,~~

~~(f) preparing a single stranded DNA molecule from said RNA-DNA duplex of (e) by~~
~~hydrolyzing the RNA transcript of said RNA-DNA duplex using ribonuclease H,~~

~~(g) forming a doubled stranded DNA molecule comprising the single stranded DNA~~
~~molecule of (f) and a complementary DNA molecule thereto, said doubled stranded DNA~~
~~molecule is formed by synthesizing a second DNA molecule complementary to at least a part of~~
~~said single stranded DNA molecule of (f) using (1) said second oligonucleotide primer to prime~~
~~the synthesis of said second DNA molecule, wherein said second primer further comprises an~~

~~RNA-transcriptable promoter sequence at its 5'-end, (2) DNA-dependent DNA-polymerase, and (3) the single-stranded DNA molecule of (f) as a template, to thereby form a double-stranded DNA molecule;~~

~~(h) — forming an RNA transcription product from said double-stranded DNA molecule of (g) using RNA polymerase, wherein RNA transcription is primed from the RNA-transcriptable promoter sequence; and~~

~~(i) — repeating steps (e) to (h) using said RNA transcription product of (h) as a template for the formation of the RNA-DNA duplex of (e).~~

14. (currently amended) A method for determining whether a selected DNA molecule encodes a gene expression region, said method comprising:

(Aa) obtaining RNA transcripts from an organism which comprises said selected DNA molecule, and

(Bb) screening said RNA transcripts for an RNA transcript corresponding ~~that is~~ complementary to a selected portion of said selected DNA molecule, wherein the nucleotide sequence of said selected portion of said selected DNA molecule is known, to thereby determine whether said selected portion of said selected DNA molecule encodes a gene expression region, wherein said screening comprises:

(ie) amplifying the RNA transcripts using a first oligonucleotide primer and a second oligonucleotide primer, wherein said first primer is complementary to a sequence of at least 10 continuous nucleotides located at or near the 3'-end of said selected portion of said selected DNA molecule, and said second primer is homologous to a sequence of at least 10 continuous nucleotides located at or near the 5'-end of said selected portion of

said selected DNA molecule, and wherein said amplifying is in a reaction solution comprising an oligonucleotide probe that specifically binds to said amplification product, wherein said probe is labeled with an intercalating fluorescence dye and said probe consists of a sequence that is not complementary to either the first or second oligonucleotide primer, and

(~~ii~~) detecting an amplification product of (ie) corresponding complementary to said selected portion of said selected DNA molecule by measuring a change in a fluorescence characteristic of the reaction solution after the amplification, to thereby screen said RNA transcripts for an RNA transcript that corresponds to said selected portion of said selected DNA molecule, and

(C) repeating (A) and (B) on at least one other selected portion of said selected DNA molecule.

15. (previously presented) The method according to claim 14, wherein said probe is complementary to at least a portion of the sequence of said amplification product, and wherein said probe is labeled with an intercalating fluorescence dye that has a differential fluorescence characteristic depending on whether said probe exists in an unbound single-stranded state or in a bound duplex with said amplification product.

16. (currently amended) A method for determining which portions of a selected DNA molecule encode a gene expression region, said method comprising:

(a) obtaining RNA transcripts from an organism which comprises said selected DNA molecule,

(b) screening said RNA transcripts for an RNA transcript corresponding that is complementary to a selected portion of said selected DNA molecule, wherein the nucleotide sequence of said selected portion of said selected DNA molecule is known, to thereby determine whether said selected portion of said selected DNA molecule encodes a gene expression region, and

(c) repeating (a) and (b) on at least one other selected portion successive DNA fragments of said selected DNA molecule.